

MS sample preparation

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Updated date: Nov 12, 2021

 An abbreviated version of this protocol was published in eLIFE in Sep 2019

Proximity labeling of protein complexes and cell-type-specific organellar proteomes in *Arabidopsis* enabled by TurboID

DOI: 10.7554/eLife.47864

Detailed protocol

MS sample prep

- Wash the beads 2x with 1ml 50mM Tris pH 7.5 (transfer to new tube with first wash)
- Wash the beads 2x with 1ml Urea wash buffer 2 (50mM Tris pH 7.5, 2M Urea)
- Remove the buffer and add 80µl Trypsin buffer (50mM Tris pH 7.5, 1M Urea, 1mM DTT, 0.4µg Trypsin)
- Incubate for 3h at 25°C with shaking
- Transfer the supernatant to a fresh tube
- Wash the beads 2x with 60µl ½ Urea wash buffer 2 (50mM Tris pH 7.5, 1M Urea) and combine the supernatants with the Trypsin digest supernatant
- Reduce the eluate by adding DTT to a final concentration of 4mM (1.6µl 500mM DTT to 200µl eluate) and incubating at 25°C for 30min with shaking
- Alkylate the eluate by adding Iodoacetamide to a final concentration of 10mM (4µl 500mM IAM to 200µl eluate) and incubating at 25°C for 45min with shaking
- Add 0.5µg Trypsin (0.5µl of 1µg/µl stock) and complete the digestion by incubating at 25°C over night (14.5h) with shaking
- Add formic acid to a final concentration of ~ 1% (2.05µl 100% formic acid)
- Desalt samples on C18 tips (all pipetting steps very slow and without getting air into the column material!)
 - Prepare a 0.65ml tube with 200µl buffer B2 (0.1% formic acid, 50% acetonitrile) for the elution
 - Activate a 100µl C18 desalting tip (2x): Aspirate 200µl buffer B2 and discard solvent
 - Equilibration (4x): Aspirate 200µl buffer A2 (0.1% formic acid) and discard solvent
 - Peptide binding (8x): Aspirate and dispense the peptide sample
 - Washing (10x): Aspirate 200µl buffer A2 and discard solvent
 - Elution: Aspirate 200µl buffer B2 from the prepared tube and pipette up and down (8x)
 - Dry the peptides in the Speed Vac

How to cite: (Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Mair, A. and Bergmann, D. (2021). MS sample preparation. Bio-protocol Preprint. [bio-protocol.org/prep1439](https://doi.org/10.21203/rs.3.rs-1000000/v1).
2. Mair, A., Xu, S., Branon, T. C., Ting, A. Y. and Bergmann, D. C. (2019). Proximity labeling of protein complexes and cell-type-specific organellar proteomes in *Arabidopsis* enabled by TurboID. eLIFE. DOI: [10.7554/eLife.47864](https://doi.org/10.7554/eLife.47864)

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